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Mineral nutrition and tolerance to *Colletotrichum* spp. of Andean blackberry (*Rubus glaucus* Benth.) in nursery[1]

Nutrición mineral y tolerancia a *Colletotrichum* spp. de mora andina (*Rubus glaucus* Benth.) en vivero

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Abstract

Introduction. Several diseases affect the Andean blackberry cultivation in Colombia, where anthracnose (*Colletotrichum* spp.) stands out for generating total losses. **Objective.** To estimate the doses of N, P, K, and Ca that allow greater tolerance to *C. gloeosporioides* strain 52 in Andean blackberry seedlings. **Materials and methods.** The experiment was carried out in 2017, in a greenhouse located in Mosquera in the department of Cundinamarca (Colombia), with seedlings sown in a substrate based on a 3: 1 mixture of peat and rice husks. A randomized complete block design with a central composite arrangement of 25 treatments and 15 seedlings were used per experimental unit. The inoculation of plant material was carried out 53 days after had begun the application of the treatments, with mycelial discs at a concentration of 9.53×10^4 conidia. The severity percentage (Sev), incubation period (IP), disease development rate (r), plant height (PLH), stem diameter (StD), leaf area (LA), chlorophyll content index (CCI), dry weight of the aerial part, and concentration of nutrients were measured. The analysis of variance with Tukey's test ($p<0.05$), correlation analysis, generation of response surface models, and principal component analysis with the t-test ($p<0.05$) was carried out. **Results.** The severity percentage showed significant differences in the plants that received the fertilization treatments, the allometric variables, and chlorophyll content index were negatively and significantly related with the severity percentage. Simple linear effects models and interaction between elements and four components were obtained, which explained 63 % of the observed variability. **Conclusions.** The doses of 55, 3, 14, and 9 g plant of N, P_2O_5, K_2O, and CaO, respectively, allowed a higher tolerance of plants against strain 52, as well as higher values in growth variables.

Keywords: optimal dose, plant nutrition, response surface, integrated management, plant health.

Resumen

Introducción. El cultivo de mora en Colombia es afectado por varias enfermedades, donde sobresale la antracnosis (*Colletotrichum* spp.), que puede generar pérdidas totales. **Objetivo.** Estimar las dosis de N, P, K y Ca que permitan mayor tolerancia a *C. gloeosporioides* cepa 52 en plántulas de mora. **Materiales y métodos.** El experimento se realizó en 2017 en un invernadero ubicado en Mosquera en el departamento de Cundinamarca (Colombia), con plántulas sembradas en sustrato a base de la mezcla 3:1 turba y cascarilla de arroz. Se utilizó un diseño experimental de bloques completos al azar con arreglo compuesto central de veinticinco tratamientos y quince plántulas por unidad experimental. El material vegetal se inoculó 53 días después de iniciada la aplicación de los tratamientos, con discos de micelio a una concentración de $9,53\times10^4$ conidias. Se registró el porcentaje de severidad (Sev), período de incubación (PI) y tasa de desarrollo de la enfermedad (r); altura de planta (AP), diámetro del tallo (StD), área foliar (AF), índice de contenido de clorofilas (ICC), peso seco de la parte aérea y concentración de nutrientes. Se realizó análisis de varianza con prueba Tukey ($p\leq0,05$), análisis de correlación, generación de modelos de superficie de respuesta y análisis de componentes principales con prueba t ($p\leq0,05$). **Resultados.** El porcentaje de severidad mostró diferencias significativas en las plantas que recibieron los tratamientos de fertilización; las variables alométricas y el índice de contenido de clorofilas se correlacionaron negativa y significativamente con el porcentaje de severidad. Se obtuvieron modelos de efectos lineales simples e interacción entre elementos y cuatro componentes que explicaron 63 % de la variabilidad observada. **Conclusiones.** Las dosis de 55, 12, 14 y 9 g/planta de N, P_2O_5, K_2O y CaO, respectivamente, permitieron mayor tolerancia de las plantas de mora frente a *C. gloeosporioides* cepa 52, así como los valores más altos en variables de crecimiento.

Palabras clave: dosis óptima, nutrición de plantas, superficie de respuesta, manejo integrado, sanidad vegetal.

Introduction

The area harvested with Andean blackberry (*Rubus glaucus* Benth.) in Colombia at the end of 2018 increased to 13,585 ha, reaching a production of 129,977 t with a national yield of 8.8 t ha^{-1}. In the country, this crop is considered as a family farming productive system, being a main crop in the departments of Cundinamarca, Santander, Antioquia, Valle de Cauca, and the Eje Cafetero region (coffee-growing area) (Agronet, 2021).

Many species of this genus are exposed to various phytosanitary problems caused by bacteria, fungi, oomycetes, nematodes, phytoplasmas, viruses, and insects that can decrease their profitability and affect the farmers' income (Martin et al., 2017). In the case of the reported diseases, anthracnose stands out (Saldarriaga-Cardona et al., 2017) because it's associated with eight fungi *Colletotrichum* species (Afanador-Kafuri et al., 2014). This fungi complex is the most limiting since it generates losses of approximately 50 %, which can be 100 % when prevention measures aren't used (Saldarriaga-Cardona et al., 2008).

The removal of affected plant material, harvesting ripe fruits, sanitary and shape prunings, selective control of weeds, and the management of planting distances are practices suggested for the management of this disease (Saldarriaga-Cardona et al., 2017). Additionally, alternative methods to chemical management have been proposed, based on the use of biocontrollers, application of plant extracts (Gaviria-Hernández et al., 2013; Hincapié Echeverri et al., 2017), and establish tolerant genotypes (López-Vásquez et al., 2013).

The supply of fertilizers for plants contributes to the decrease or increase of phytosanitary problems (Gupta et al., 2017) due to the activation of tolerance factors and/or predisposition in plants by nutritional imbalances (Chaboussou, 1967). The mechanisms that lead to decreased disease development caused by various pathogens are complex (Walters & Bingham, 2007), and include effects on the growth and development of plants (Prabhu et al.,

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2007), resistance mechanisms (Mithöfer & Maffei 2017), and direct effects of the elements on pathogens (Borkow & Gabbay, 2009; Deshmukh et al., 2012; Núñez-Zofío et al., 2011).

In line with the above, the nitrogen (N) is involved in plant stress responses, but its roles in host-pathogen interactions are complex. The N fertilization can increase plant disease incidence, for example, downy mildew, powdery mildew, leaf rust, and stem rot. However, the opposite effects have been reported for take-all, grey mould, and leaf spot disease. In this case, the outcomes cannot be associated with a given host or different infection strategy, whether necrotrophic or (hemi) biotrophic, or if a foliar or root-infecting pathogen (Sun et al., 2020).

For the case of phosphorus (P), the effect on diseases isn't always consistent, and therefore, balanced soil fertility is important for reducing plant stress, improving the level of resistance, and decreasing the diseases severity. The P fertilization effectively reduces the incidence of Pythium root rot, smut, blotch, powdery mildew, and take-all in wheat, root rot in barley, corn smut, bacterial leaf blight, and yellow and blast disease in rice, downy mildew, blue mould, tobacco leaf curl virus, and pod and stem blight in soya bean (Singh, 2015).

Potassium (K) is involved in many cellular processes and therefore influences disease severity. The amount of the plants natural antifungal compounds at the site of infection decreases during K deficiency and has a direct role in the development of thick cuticles which acts as a physical barrier to infection or penetration by sucking insects (Singh, 2015). The maize plants resistance to Fusarium diseases increased significantly with applications potassium fertilizers (Bocianowski et al., 2016). Likewise, the K applications significantly reduced Fusarium wilt symptom expression in apple and bananas (Nowembabazi et al., 2021). In soybean, the K applications induce multiple mechanisms to improve the resistance against *Heterodera glycines* (Gao et al., 2018).

The permeability of the cell membrane is affected adversely and allows leakage of low-molecular-weight compounds from the cytoplasm to the apoplast under Ca deficiency. The above due to the Ca provides mechanical strength to the tissues and resistance to fungal infections (Singh, 2015). Studies about applications of Ca shown reduced the incidence of gray mold (*Botrytis cinerea*) in roses, *Sclerotium rolfsii* and *Pythium coloratum* in carrot, *Plasmodiophora brassicae* in cabbage, and *Fusarium oxysporum* f. sp. *lycopersici* in tomato (Elmer & Datnoff, 2014).

Despite the developments in integrated fertilization of Andean blackberry crops (Cardona et al., 2017; 2018; Cardona & Bolaños-Benavides, 2019; Castaño et al., 2008), no research about evaluating the effect of plant nutrition on the development of anthracnose has been carried out in Colombia. Consequently, the aim of this study was to estimate the optimal doses of N, P, K, and Ca that allowed greater tolerance to *C. gloeosporioides* strain 52 in Andean blackberry seedlings.

Materials and methods

Study site

The experiment was carried out in 2017, in the greenhouse of the Tibaitatá Research Center of the Colombian Corporation for Agricultural Research (AGROSAVIA), located in Mosquera in the department of Cundinamarca (Colombia), at a latitude of N 4º41'43.1349'', a longitude of W 74º12'18.7666'', and altitude of 2,600 m. Inside the greenhouse and throughout the evaluation stage, an average temperature was 19 °C and 57 % of relative humidity was registered.

Plant material

Seedlings of Andean blackberry (*Rubus glaucus* Benth.) sexually propagated, with phenotypic uniformity of 10 months of age and susceptible to anthracnose were used. These were purchased in a nursery endorsed by the

National Plant Protection Organization of Colombia. The plants were transplanted into 1,500 g plastic bags with peat and rice husk mixture, in a 3:1 ratio, previously autoclaved during a 60 min cycle.

Treatments' design

Following the methodology used by Cardona et al. (2018), a central composite design was used (Equation 1), through which 25 treatments were defined (Table 1).

$$2^k + 2 * k + 1 \qquad (1)$$

Where: k is the number of factors or nutrients (i.e., N, P, K, and Ca).

The nutrients were dosed and applied such as nutritional solutions prepared from HNO_3, $CaCl{\cdot}2H_2O$, KCl, $(NH_4)_2HPO_4$, $MgSO_4{\cdot}7H_2O$, $Ca(NO_3)_2{\cdot}4H_2O$, KNO_3, K_2SO_4, and NaOH (to regulate pH), using concentrations of Mg, S, and microelements from the nutrient solution of Hoagland and Arnon (1950). The frequency of application was every four days for four months, with an application volume of 44 mL of nutritive solution per plant, and the concentration of nutrients increased each month agree to the development of the plants.

Pathogen inoculation

The total duration of the experiment was 122 days. For disease assessment, 53 days after the application of the different doses of nutrients, one stem per plants were artificially inoculated with mycelial discs agar of *C. gloeosporioides* strain 52 belongs to the collection of microorganisms of AGROSAVIA (isolated and molecular characterized), at a concentration of approximately $9.53x10^4$ conidia. For the latter, the number of conidia was calculated by agitation in deionized water and subsequent cell count of a representative sample of the mycelial disk, in a Neubauer chamber.

Disease severity and allometric variables

For the disease evaluation on the plants inoculated with strain 52 of *C. gloeosporioides*, a diagrammatic severity scale was developed in Andean blackberry stems, using as a reference the scale proposed by López-Vásquez et al. (2013) (Figure 1). Subsequently, the severity of the disease (Sev) was estimated using equation 2.

$$Sev = \frac{\sum(n*b)}{N} \qquad (2)$$

Where *Sev* is the percentage of tissue affected, *n* is the number of sampling units classified in each grade; *b* is the severity percentage recorded in each sampling unit, and *N* is the total number of observed sampling units.

Once the severity was estimated, the disease development rate (r) was calculated through the following equation (Castaño Zapata, 2002) (Equation 3):

$$r = \frac{1}{t_1 - t_0}\left(Log_e \frac{X_1}{1 - X_1} - Log_e \frac{X_0}{1 - X_0}\right) \qquad (3)$$

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Where r is the development rate, t_1 is the final time, t_0 is the initial time, X_1 is the final severity percentage, and X_0 is the initial severity percentage.

Table 1. Fertilization treatments (grams of N, P, K, and Ca per plant) used in the nursery phase to estimate the tolerance to *C. gloeosporioides* strain 52 in Andean blackberry (*Rubus glaucus* Benth.) seedlings. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Cuadro 1. Tratamientos de fertilización utilizados (gramos de N, P, K y Ca por planta) en fase de vivero para estimar la tolerancia a *C. gloeosporioides* cepa 52 en plántulas de mora (*Rubus glaucus* Benth.). Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Treatments	30 days after sowing				60 days after sowing				90 days after sowing				Total			
	N	P_2O_5	K_2O	CaO	N	P_2O_5	K_2O	CaO	N	P_2O_5	K_2O	CaO	N	P_2O_5	K_2O	CaO
1	1.6	1.2	3.8	1.7	4.1	2.6	4.7	2.8	10.3	8.2	15.5	7.5	16	12	24	12
2	6.6	1.2	3.8	1.7	10.5	2.6	4.7	2.8	30.9	8.2	15.5	7.5	48	12	24	12
3	1.6	3.6	3.8	1.7	4.1	11.7	4.7	2.8	10.3	41.7	15.5	7.5	16	57	24	12
4	6.6	3.6	3.8	1.7	10.5	11.7	4.7	2.8	30.9	41.7	15.5	7.5	48	57	24	12
5	1.6	1.2	4.7	1.7	4.1	2.6	16.4	2.8	10.3	8.2	50.9	7.5	16	12	72	12
6	6.6	1.2	4.7	1.7	10.5	2.6	16.4	2.8	30.9	8.2	50.9	7.5	48	12	72	12
7	1.6	3.6	4.7	1.7	4.1	11.7	16.4	2.8	10.3	41.7	50.9	7.5	16	57	72	12
8	6.6	3.6	4.7	1.7	10.5	11.7	16.4	2.8	30.9	41.7	50.9	7.5	48	57	72	12
9	1.6	1.2	3.8	2.2	4.1	2.6	4.7	6.2	10.3	8.2	15.5	19.6	16	12	24	28
10	6.6	1.2	3.8	2.2	10.5	2.6	4.7	6.2	30.9	8.2	15.5	19.6	48	12	24	28
11	1.6	3.6	3.8	2.2	4.1	11.7	4.7	6.2	10.3	41.7	15.5	19.6	16	57	24	28
12	6.6	3.6	3.8	2.2	10.5	11.7	4.7	6.2	30.9	41.7	15.5	19.6	48	57	24	28
13	1.6	1.2	4.7	2.2	4.1	2.6	16.4	6.2	10.3	8.2	50.9	19.6	16	12	72	28
14	6.6	1.2	4.7	2.2	10.5	2.6	16.4	6.2	30.9	8.2	50.9	19.6	48	12	72	28
15	1.6	3.6	4.7	2.2	4.1	11.7	16.4	6.2	10.3	41.7	50.9	19.6	16	57	72	28
16	6.6	3.6	4.7	2.2	10.5	11.7	16.4	6.2	30.9	41.7	50.9	19.6	48	57	72	28
17	1.0	3.5	3.8	1.7	1.5	5.8	10.4	3.9	6.5	25.7	33.8	14.4	9	35	48	20
18	5.2	3.5	3.8	1.7	10.8	5.8	10.4	3.9	39.0	25.7	33.8	14.4	55	35	48	20
19	2.3	0.5	3.8	1.7	7.7	0.7	10.4	3.9	22.0	1.8	33.8	14.4	32	3	48	20
20	2.3	5.0	3.8	1.7	7.7	17.0	10.4	3.9	22.0	44.0	33.8	14.4	32	66	48	20
21	2.3	3.5	1.9	1.7	7.7	5.8	2.8	3.9	22.0	25.7	9.3	14.4	32	35	14	20
22	2.3	3.5	9.5	1.7	7.7	5.8	16.0	3.9	22.0	25.7	56.5	14.4	32	35	82	20
23	2.3	3.5	3.8	1.5	7.7	5.8	10.4	2.1	22.0	25.7	33.8	5.4	32	35	48	9
24	2.3	3.5	3.8	5.5	7.7	5.8	10.4	6.5	22.0	25.7	33.8	19.0	32	35	48	31
25	2.3	3.5	3.8	1.7	7.7	5.8	10.4	3.9	22.0	25.7	33.8	14.4	32	35	48	20

Subsequently, the incubation period (IP) was obtained and described as the time elapsed between the inoculation of the tissue until the manifestation of the first anthracnose symptoms.

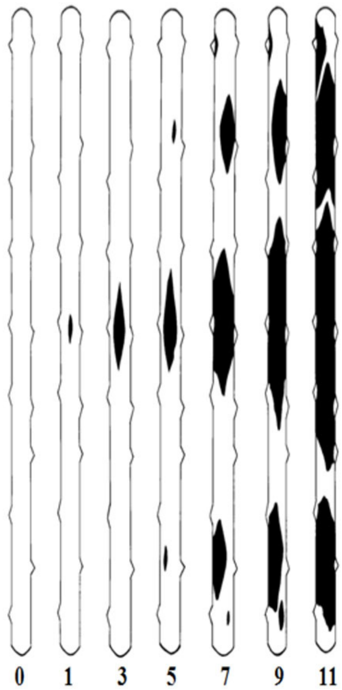

Figure 1. Anthracnose severity scale of Andean blackberry (*Rubus glaucus* Benth.) stems inoculated with *C. gloeosporioides* strain 52 (modified from López-Vásquez et al., 2013). Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

***0** = no apparent injury or 0 % of the affected tissue, **1** = 1 % of the affected tissue, **3** = 5 % of the affected tissue, **5** = 10 % of the affected tissue, **7** = 25 % of the affected tissue, **9** = 50 % of the affected tissue, and **11** = 85 % of the affected tissue.

Figura 1. Escala de severidad de la antracnosis en tallos de mora (*Rubus glaucus* Benth.) inoculados con *C. gloeosporioides* cepa 52 (modificado de López-Vásquez et al., 2013). Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

***0** = sin lesión aparente o 0 % del tejido afectado, **1** = 1 % del tejido afectado, **3** = 5 % del tejido afectado, **5** = 10 % del tejido afectado, **7** = 25 % del tejido afectado, **9** = 50 % del tejido afectado y **11** = 85 % del tejido afectado.

The allometric variables recorded are the following: a) plant height (PLH) using a flexometer, and recording the value in cm from the base of the stem to the apex of the plant, b) stem diameter (StD) measured one centimeter from the base, and using a digital caliper with a millimeter-scale, c) chlorophyll content index (CCI) using a portable chlorophyll meter, taking three readings on a fully expanded leaflet of the third trifolium, d) leaf area (LA) calculated by analysis of digital images captured with a camera and processed in the free software ImageJ 1.50i (National Institutes of Health, USA) with a scale in cm^2, e) concentration of macro and micronutrients (N, P, K, Ca, Mg, Na, S, B, Cu, Fe, Mn y Zn) in plant tissue of plants surviving inoculation with strain 52, according to the protocols of the analytical chemistry laboratory of AGROSAVIA, under NTC ISO/IEC 17025, and f) dry weight (DW) of the aerial part by oven drying at 75 °C for 48 h and using an analytical balance.

Re-isolation of *C. gloeosporioides* strain 52 and biological test

The re-isolation of *C. gloeosporioides* strain 52 and biological test were carried out in the Agricultural Microbiology laboratory of the Tibaitatá Research Center of AGROSAVIA. Samples were taken from the middle third of symptomatic stems about 10 cm, which were disinfected with distilled water, 2.5 % sodium hypochlorite, and 70 % alcohol. In a laminar flow chamber, stem fragments were seeded in Petri dishes containing PDA culture medium previously acidified with 50 % lactic acid. Petri dishes were sealed and incubated at 25 °C. Subsequently, a peal of the initial isolates was made to observe the macroscopic characteristics in the acidified PDA medium, as well as microscope setups for the observation of reproductive structures according to the description given by Bailey and Jeger (1992). This process needed the reactivation of strain 52 used in the inoculation as a comparison standard.

To confirm that the fungi re-isolated fungi corresponded to the strain used in the initial inoculation, an *in vitro* sensitivity test was performed, using fungicides benomyl (*mechanism of action:* disruption of tubulin polymerization) and copper hydroxide (*mechanism of action:* denatures proteins and inactivates enzymes and coenzymes), with a concentration of 250 mg kg^{-1} and 2460 mg kg^{-1} in acidified PDA medium, respectively. For this, 0.5 mm PDA discs with the strain 52 were used, and the strain 1164 of *C. acutatum* isolated from the Andean blackberry crop (donated from the AGROSAVIA plant pathology laboratory), which was used as a control, and the isolates from the tissue collected in the evaluated treatments were used. The fungicides were used to differentiate the *C. acutatum* and *C. gloeosporioides* species, in terms of their macroscopic characteristics and their growth speed in the media, as reported by Gaviria-Hernández et al. (2013). Finally, the growth measurement of the discs of each isolate was carried out since the second day of sowing until day 10.

Experimental design and statistical analysis

A randomized complete block experimental design (three blocks) with 25 treatments and 5 seedlings per experimental unit was used, for a total of 75 experimental unit and 375 seedlings. Variables related to the severity percentage (Sev), incubation period (IP), and disease development rate (r) were subjected to analysis of variance (ANOVA) and Tukey's test ($p<0.05$) to detect significant differences between treatments. Additionally, a correlation analysis was performed between the severity percentage and the allometric variables recorded.

Likewise, regression models were performed, selecting significant terms ($p<0.05$) from a model with linear and multiple effects for the doses of N, P, K, and Ca on the percentage of severity and the allometric variables. A principal component analysis (PCA) was carried out with the results of concentration of macro and micronutrients in plant tissue, Sev, and allometric variables. Furthermore, a multivariate analysis of variance (MANOVA) and a t-test ($p<0.05$) were also performed.

Regarding the effect of the two fungicides on mycelial growth, it was evaluated independently, using a randomized complete design (CRD), composed of three repetitions.

Data processing was carried out using the R software 4.1.2 (R Foundation for Statistical Computing).

Results

Disease severity

The disease severity (Sev) percentage showed significant differences ($p<0.0001$) in the plants that received the fertilization treatments (Table 2). The results showed tolerance to the *C. gloeosporioides* strain 52 in the treatment 23. It was corroborated because this treatment also had given the lower Sev and r and longer IP. On the other hand, the plants that received the doses of treatments 11, 15, and 24, exhibited higher susceptibility when showing higher Sev and r, and shorter IP (Table 2). Plants that received the remaining treatments expressed intermediate trends, registering ranges of Sev, IP, and r, between 18 % to 90 %, 9 to 21 days, and 0.0256 to 0.0410, respectively (Table 2).

Leaf area (LA), plant height (PLH), chlorophyll content index (CCI), and stem diameter (StD) were negatively and significantly related ($p<0.0001$) with the percentage of Sev, being CCI and StD the ones with the highest

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Table 2. Severity percentage (Sev), incubation period (IP), and development rate (r) of *C. gloeosporioides* strain 52 in stems of Andean blackberry (*Rubus glaucus* Benth.) with 25 fertilization treatments. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Cuadro 2. Porcentaje de severidad (Sev), período de incubación (IP) y tasa de desarrollo (r) de *C. gloeosporioides* cepa 52, en tallos de mora andina (*Rubus glaucus* Benth.) con 25 tratamientos de fertilización. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Treatments	Sev (%)	IP (days)	r
1	29.0 bcde*	15.0**	0.0268**
2	22.1 cde	15.0	0.0271
3	87.7 abc	9.00	0.0412
4	40.2 abcde	9.00	0.0269
5	28.7 bcde	9.00	0.0251
6	31.2 bcde	21.0	0.0272
7	90.4 ab	9.00	0.0410
8	38.4 abcde	21.0	0.0319
9	53.6 abcde	15.0	0.0340
10	41.5 abcde	15.0	0.0293
11	99.0 a	9.00	0.0538
12	28.3 bcde	15.0	0.0285
13	69.6 abcde	15.0	0.0347
14	60.1 abcde	15.0	0.0342
15	100 a	9.00	0.0553
16	72.0 abcde	9.00	0.0356
17	80.8 abcd	9.00	0.0370
18	28.1 bcde	15.0	0.0305
19	36.1 abcde	15.0	0.0275
20	57.0 abcde	21.0	0.0354
21	18.2 ed	15.0	0.0256
22	59.2 abcde	15.0	0.0433
23	7.58 e	21.0	0.0237
24	100 a	9.00	0.0549
25	83.4 abcd	9.00	0.0389

*Averages with different letters indicate a significant difference according to the Tukey´s test ($p<0.05$). **not significant. / *Los promedios con letras diferentes indican una diferencia significativa según la prueba de Tukey ($p<0,05$). **no significativo.

correlation coefficient (Table 3). Regarding the allometric variables, it was possible observed higher positive and significant relationships ($p<0.0001$) between PLH with CCI, StD, and LA (Table 3).

The *C. gloeosporioides* re-isolated of inoculated plants that developed symptoms, exhibited gray to olive green and grayish green colorations, while microscopically, ovoid to oblong conidia with slight constriction and nailed to irregular appressoria were observed. These characteristics were similar between the re-isolates and the strain 52 used for the initial inoculation (Figure 2).

The benomyl fungicide presented 100 % of inhibition on the fungi re-isolated from the treatments, as well as for the *C. gloeosporioides* strain 52 used in the initial inoculation. For *C. acutatum* strain 1164, a reduced inhibitory effect on mycelial growth, which reached a maximum diameter of 37.3 mm. However, the fungicide based on copper hydroxide did not present differences in mycelial growth between treatments, strain 52 and strain 1164, achieving 100 % inhibition in all cases (Figure 3).

Table 3. Correlation matrix between severity percentage, chlorophyll content index, and allometric variables of Andean blackberry (*Rubus glaucus* Benth.) plants inoculated with C. *gloeosporioides* strain 52. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Cuadro 3. Matriz de correlación entre porcentaje de severidad, índice de contenido de clorofila y variables alométricas de plantas de mora andina (*Rubus glaucus* Benth.) inoculadas con *C. gloeosporioides* cepa 52. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

	Sev (%)	**CCI**	**PLH (cm)**	**StD (mm)**
CCI	-0.711*			
PLH (cm)	-0.638	0.805		
StD (mm)	-0.776	0.711	0.792	
LA (cm^2)	-0.497	0.746	0.584	0.512

*Pearson's correlation coefficients ($p < 0.05$). **Sev:** severity percentage, **CCI:** chlorophyll content index, **PLH:** plant height, **StD:** stem diameter, and **LA:** leaf area. / *Coeficientes de correlación de Pearson ($p < 0.05$). **Sev:** porcentaje de severidad, **CCI:** índice de contenido de clorofila, **PLH:** altura de la planta, **StD:** diámetro del tallo y **LA:** área foliar.

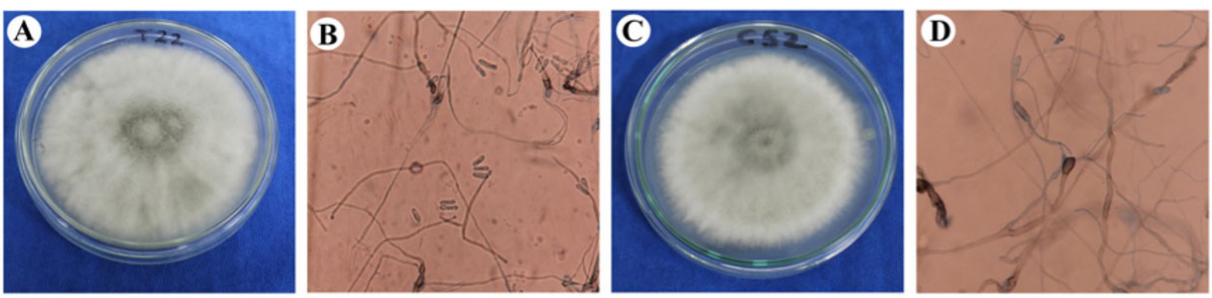

Figure 2. Colony (A), conidia and appressoria (B) of *C. gloeosporioides* re-isolated of stems of Andean blackberry treated with fertilization treatment 22 and colony (C), conidia and appressoria (D) of *C. gloesporioides* strain 52 used in the inoculations. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 2. Colonia (A), conidios y apresorios (B) de *C. gloeosporioides* re-aislados de tallos de mora andina tratados con el tratamiento 22 de fertilización y colonia (C), conidios y apresorios (D) de *C. gloesporioides* cepa 52 utilizados en las inoculaciones. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Response surface models

In accordance with the ANOVA (data not shown), the registered variables were significantly influenced ($p<0.05$) by the different doses of nutrients applied, except for K, which economically discards it, suggesting the application of the lowest dose for this element. By adjusting the generated models, simple linear effects and interaction between elements were observed for Sev, CCI, and LA, meanwhile, for variables PLH, and StD, a simple linear effect was observed (Table 4).

A positive and linear individual effect of N, P, and Ca was evidenced on Sev. However, the N*P, N*Ca, and P*Ca interaction had a negative effect (Table 4). In the CCI variable, a linear positive effect was found with N and linear negative with P and Ca, which became negative and positive in the interactions between N*Ca and P*Ca, respectively (Table 4). Regarding to LA, a linear and positive effect was found with N and Ca, which became negative and significant when there was an interaction between two elements (Table 4).

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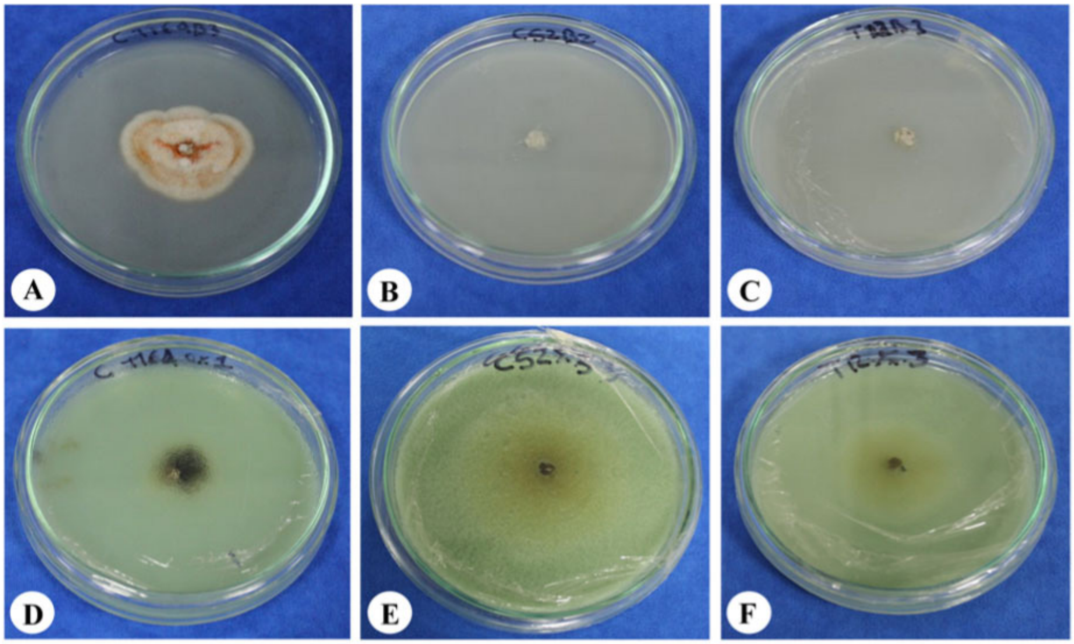

Figure 3. Growth of *Colletotrichum* spp. in PDA medium + benomyl (A, B, and C) and PDA medium + copper hydroxide (D, E, and F). *C. acutatum* strain 1164 (A and D), *C. gloeosporioides* strain 52 used in the inoculation (B and E), *C. gloeosporioides* re-isolated from stems in treatment 12 (C and F). Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 3. Crecimiento de *Colletotrichum* spp. en medio PDA + benomil (A, B y C) y medio PDA + hidróxido de cobre (D, E y F). *C. acutatum* cepa 1164 (A y D), *C. gloeosporioides* cepa 52 utilizada en la inoculación (B y E), *C. gloeosporioides* reaislado de tallos en el tratamiento 12 (C y F). Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Table 4. Coefficients and associated error of the significant terms ($p<0.05$) of the regression model for severity percentage, chlorophyll content index, and allometric variables of Andean blackberry (*Rubus glaucus* Benth.) plants inoculated with *C. gloeosporioides* strain 52. Corporacion Colombiana de Investigacion Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Cuadro 4. Coeficientes y error asociado de los términos significativos ($p<0,05$) del modelo de regresión para porcentaje de severidad, índice de contenido de clorofila y variables alométricas de plantas de mora andina (*Rubus glaucus* Benth.) inoculadas con *C. gloeosporioides* cepa 52. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Factor	**Sev (%)**		**CCI**		**PLH (cm)**		**StD (mm)**		**LA (cm²)**	
	Estimator	**Error**	**Estimator**	**Error**	**Estimator**	**Error**	**Estimator**	**Error**	**Estimator**	**Error**
Intercept	-28.30	33.79	3.550	2.890	21.57	6.979	1.792	0.129	-114.3	153.3
N	0.438	0.836	0.201	0.067	0.838	0.199	0.012	0.004	16.20	4.370
P	1.960	0.645	-0.093	0.048						
Ca	2.799	1.460	-0.051	0.136					2.125	7.221
N*P	-0.032	0.012								
N*Ca	-0.012	0.034	-0.006	0.003					-0.438	0.206
P*Ca	-0.020	0.024	0.003	0.002						
R2	0.61		0.52		0.41		0.29		0.69	

Sev: severity percentage, **CCI:** chlorophyll content index, **PLH:** plant height, **StD:** stem diameter, and **LA:** leaf area. / **Sev:** porcentaje de severidad, **CCI:** índice de contenido de clorofila, **PLH:** altura de la planta, **StD:** diámetro del tallo y **LA:** área foliar.

The response surface analysis showed an increase in disease severity with the increase in the dose of P and Ca. However, this effect was reduced with the application of the highest dose of N and the combination of the lowest doses of P and Ca (Figures 4, 5, and 6).

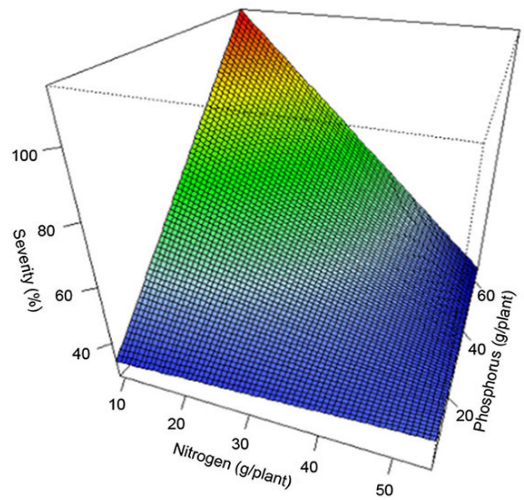

Figure 4. Response surface for the severity percentage of *C. gloeosporioides* strain 52 inoculated in Andean blackberry (*Rubus glaucus* Benth.) stems with the N*P interaction. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 4. Superficie de respuesta para el porcentaje de severidad de *C. gloeosporioides* cepa 52 inoculada en tallos de mora andina (*Rubus glaucus* Benth.) con la interacción N*P. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

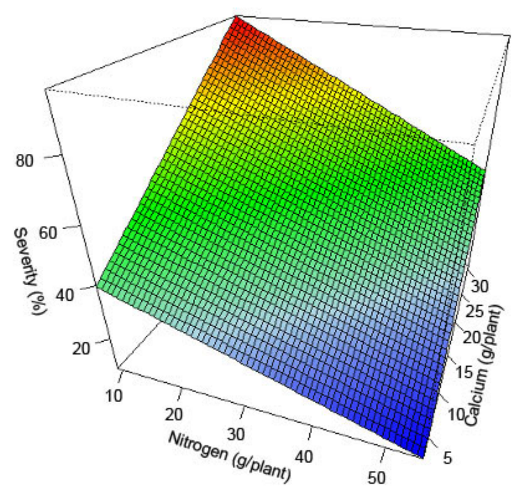

Figure 5. Response surface for the severity percentage of *C. gloeosporioides* strain 52 inoculated in Andean blackberry (*Rubus glaucus* Benth.) stems with the N*Ca interaction. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 5. Superficie de respuesta para el porcentaje de severidad de *C. gloeosporioides* cepa 52 inoculada en tallos de mora andina (*Rubus glaucus* Benth.) con la interacción N*Ca. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

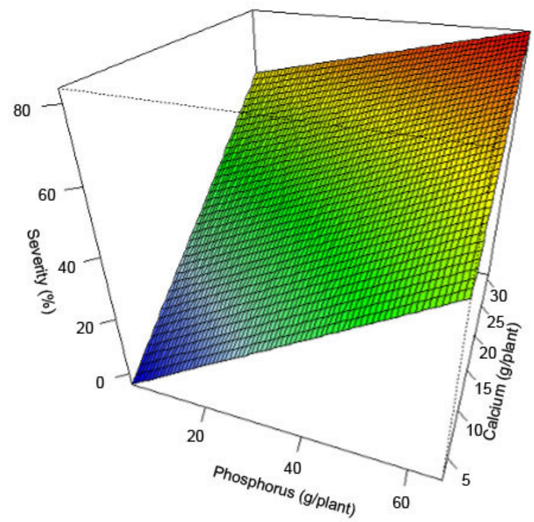

Figure 6. Response surface for the severity percentage of *C. gloeosporioides* strain 52 inoculated in Andean blackberry (*Rubus glaucus* Benth.) stems with the P*Ca interaction. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 6. Superficie de respuesta para el porcentaje de severidad de *C. gloeosporioides* cepa 52 inoculada en tallos de mora andina (*Rubus glaucus* Benth.) con la interacción P*Ca. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

In terms of CCI, the response surfaces analysis showed an increase with the application of the highest dose of N, and the combination of the lowest doses of P and Ca (Figure 7 and Figure 8). Finally, LA variable exhibited

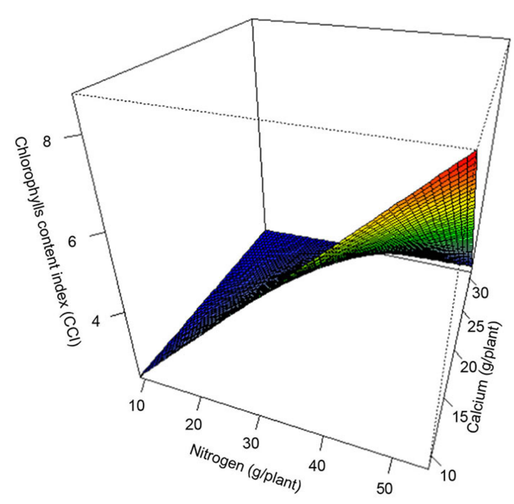

Figure 7. Response surface for the chlorophyll content index (CCI) in *C. gloesporioides* strain 52 inoculated in Andean blackberry (*Rubus glaucus* Benth.) plants with the N*Ca interaction. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 7. Superficie de respuesta para el índice de contenido de clorofila (CCI) con *C. gloeosporioides* cepa 52 inoculada en plantas de mora andina (*Rubus glaucus* Benth.) con la interacción N*Ca. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

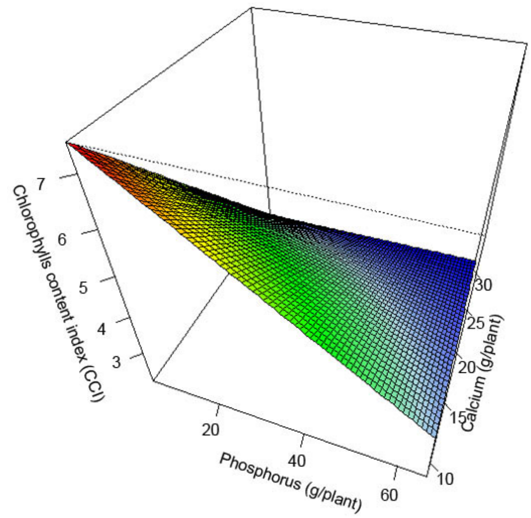

Figure 8. Response surface for the chlorophyll content index (CCI) in *C. gloesporioides* strain 52 inoculated in Andean blackberry (*Rubus glaucus* Benth.) plants with the P*Ca interaction. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 8. Superficie de respuesta para el índice de contenido de clorofila (CCI) con *C. gloeosporioides* cepa 52 inoculada en plantas de mora andina (*Rubus glaucus* Benth.) con la interacción P*Ca. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

a positive response with the maximum dose of N, an effect that decreased when Ca doses increased (Figure 9). These results highlight the importance of managing the dose of Ca, showing a maximum level of response with the application of 9 g of CaO per plant.

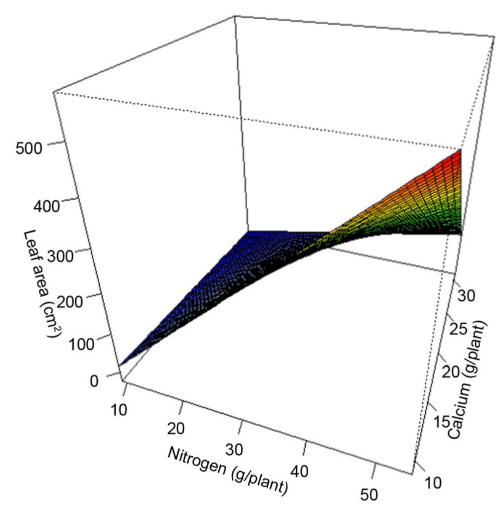

Figure 9. Response surface for the leaf area *C. gloesporioides* strain 52 inoculated in Andean blackberry (*Rubus glaucus* Benth.) plants with the N*Ca interaction. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Figura 9. Superficie de respuesta del área foliar con *C. gloeosporioides* cepa 52 inoculada en plantas de mora andina (*Rubus glaucus* Benth.) con la interacción N*Ca. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Regarding to plant height (PLH) and stem diameter (StD) in plants exposed to inoculation with strain 52, N showed a positive and linear effect, allowing plants to reach the highest values with the highest doses of this element.

After the pathogen inoculation, the StD showed a decrease in 10 of the 25 evaluated treatments, being more noticeable in treatments 24, 7, and 16, with reductions of 90 %, 45 %, and 36 %, respectively.

Finally, considering the results obtained in the response surface models, it is observed that the highest doses of N, as well as the lowest of P, K, and Ca (55 g of N, 3 g of P_2O_5, 14 g of K_2O, and 9 g of CaO) allowed to obtain a lower percentage of severity and disease development rate, as well as the highest records in growth variables.

Principal component analysis

Using a MANOVA, a significant effect (p<0.0001) of the different doses of nutrients and the variables evaluated was found, selecting four components that explained 63 % of the observed variability. In the first principal component (PC1), the variables with the greatest weight (≥0.35) that are grouped were dry weight (DW: 0.4), plant height (PLH: 0.7), leaf area (LA: 0.8), chlorophyll content index (CCI: 0.9), and stem diameter (StD: 0.5) of the plant were grouped, as well as N (0.4), Ca (-0.5), Mg (-0.7), Na (-0.7), and Cu (-0.5). In the second component (PC2), P (0.8), S (0.5), Cu (0.6), Fe (0.6), and Zn (0.8) were gathered. In the third component (PC3), disease severity (Sev: 0.7) and StD (-0.6) were grouped. The fourth component (PC4) did not show significant differences for the treatments, elements K (0.6) and B (-0.6) were gathered (Figure 10). The analysis was carried out on plants that survived the inoculation of the *C. gloeosporioides* strain 52.

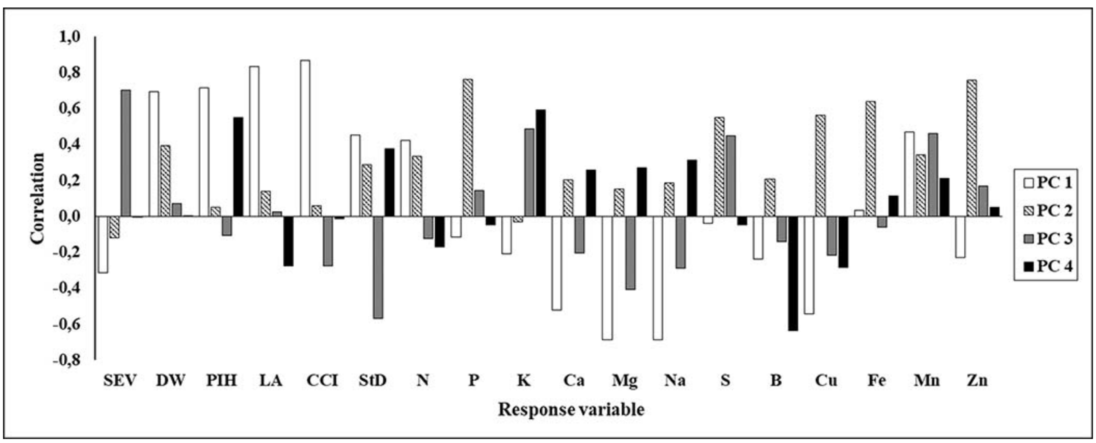

Figure 10. Correlation with significant effect (p<0.0001) among variables evaluated according to the principal components (PC) analysis in Andean blackberry (*Rubus glaucus* Benth.) plants under different nutrient doses and inoculated with *C. gloesporioides* strain 52. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

***Sev:** severity percentage, **DW:** dry weight of the aerial part, **PLH:** plant height, **LA:** leaf area; **CCI:** chlorophyll content index, and **StD:** stem diameter.

Figura 10. Correlación con efecto significativo (p<0.0001) entre variables evaluadas según los componentes principales (PC) extraídos, en plantas de mora andina (*Rubus glaucus* Benth.) bajo diferentes dosis de nutrientes e inoculada con *C. gloeosporioides* cepa 52. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

***Sev:** porcentaje de severidad, **DW:** peso seco de la parte aérea, **PLH:** altura de la planta, **LA**: área foliar, **CCI:** índice de contenido de clorofila y **StD:** diámetro del tallo.

At the treatment level, variables DW, PLH, LA, CCI, and StD associated with PC1, showed the highest values in plants that received doses of treatments 23, 6, and 4. These variables, were positively correlated with the contents of N, and negatively with Ca, Mg, Na, and Cu (Table 5 and Figure 10). According to the PC2, the highest concentrations of P, S, Cu, Fe, and Zn were found in plants that received treatments 8 and 5, which were positively correlated with each other (Table 5 and Figure 10). Finally, in PC3, the highest Sev was negatively correlated with StD and was higher in plants that received treatments 22 and 8.

Table 5. Comparison test for three principal components (PC1, PC2, and PC3) in Andean Blackberry (*Rubus glaucus* Benth.) plants under different nutrient doses and inoculated with *C. gloesporioides* strain 52. Colombian Corporation for Agricultural Research (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

Cuadro 5. Prueba de comparación para tres componentes principales (PC1, PC2 y PC3) en plantas de mora andina (*Rubus glaucus* Benth.) bajo diferentes dosis de nutrientes e inoculada con *C. gloeosporioides* strain 52. Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA). Mosquera, Cundinamarca, Colombia. 2017.

	PC1			PC2			PC3	
Treatment	**Mean**	***P***	**Treatment**	**Mean**	***P***	**Treatment**	**Mean**	***P***
23	2.915	a*	**8**	4.2706	a	**22**	2.0386	a
6	2.071	a	**5**	0.9956	ab	**8**	1.5344	ab
4	2.013	a	**4**	0.9654	b	**14**	1.4811	abc
8	1.535	ab	**12**	0.2859	b	**20**	1.4740	abc
2	1.444	ab	**21**	0.2219	b	**4**	0.5738	abcd
18	1.121	ab	**20**	0.2042	b	**5**	0.3621	abcd
21	0.138	ab	**9**	0.1974	b	**6**	0.3246	abcd
14	0.134	ab	**18**	0.0796	b	**19**	0.0298	abcd
1	0.471	ab	**2**	0.1825	b	**18**	0.4991	abcd
10	0.677	ab	**10**	0.3601	b	**1**	0.5381	abcd
5	0.788	ab	**22**	0.6072	b	**23**	0.6744	bcd
12	0.881	ab	**23**	0.6097	b	**10**	0.9981	bcd
22	-1.496	ab	**6**	-1.1183	b	**2**	-1.0286	bcd
19	-1.510	ab	**14**	-1.3642	b	**9**	-1.1756	d
20	-2.009	ab	**19**	-1.4377	b	**21**	-1.3790	d
9	-3.271	b	**1**	-1.5410	b	**12**	-1.4661	d

*****Averages with different letters indicate a significant difference according to the t-test ($p<0.05$). / *****Los promedios con letras diferentes indican una diferencia significativa según la prueba t ($p<0,05$).

Discussion

The plants that received the fertilization of treatment 23 presented higher tolerance to *C. gloeosporioides* strain 52 expressed as lower Sev and r and longer IP, while the plants exposed to treatment 24 exhibited greater susceptibility with higher Sev and r, and lower IP (Table 1). The only difference between treatments was the dose of Ca applied, being this three times higher in treatment 24. It is inferred that the negative response of Sev, IP, and r observed in the plants that received treatment 24, were influenced by an antagonistic effect with nutrients such as P that can predispose the plant to pathogen attacks, as stated by Cardona et al. (2018) and indicates that attention should be paid to the management of P doses in fertilization plans. In addition to the significant effect between treatments and their doses, the negative relationship of Sev with allometric variables in plants with high r can be interpreted as an adverse effect on the physiological development of plants by the pathogen. According to the latter, species that cause anthracnose generate severe damage in different crops due to their high infectivity and preference for specific tissues (Afanador-Kafuri et al., 2014; Agrios, 2005; Wharton & Diéguez-Uribeondo, 2004).

Despite advances in the diagnosis of disease-causing agents, the results obtained in the present study allows corroborating the importance of classical taxonomy and biological tests of the re-isolates in the identification of pathogenic microorganisms, as a complement to the molecular biology techniques used to characterize the species associated with to the Andean blackberry crop (Afanador-Kafuri et al., 2014). Additionally, these results coincided with those obtained by Gaviria-Hernández et al. (2013), who reported higher mycelial growth of *C. acutatum* strain 168 over *C. gloeosporioides* strain 52 in the presence of benomyl; as well as 100 % mycelial inhibition of both strains with copper hydroxide.

In accordance with the results observed in the response surface models, the application of the lowest dose of K is the most viable from economical point. In addition, the results coincided with the reported by Cardona et al. (2018) who did not find a significant effect of different doses of this nutrient on any of the allometric variables of Andean blackberry plants with thorns (*R. glaucus* Benth.) in the nursery stage. Similarly, effects of the K doses were not observed in the height of *Corymbia citriodora* plants in greenhouse conditions, even when they were cultivated in soil with a low content of this nutrient (Lopo de Sá et al., 2014). The surface models for Sev showed that doses of Ca must be low to potentiate the positive effects of the interaction between N and P, as stated by Cardona et al. (2018).

The results in this study showed a reduction of Sev with the application of the highest dose of N and the lowest of P, which it's differs from the results obtained by Nam et al. (2006). These researchers report that high concentrations of N and K contribute to the increase in the severity of *C. gloeosporioides* strain CGF43 in strawberry plants cv. Nyoho, while high doses of P and Ca decreased the negative effect of the fungus. On the other hand, report that high doses of N reduced the intensity of *C. musae* in fruits of 'Prata Anã' banana (Alves Freitas et al., 2019), and high doses of N combined with P reduced the incidence of *Cercospora* sp. in corn (Okori et al., 2004).

According to the above, although disease management based on the reduction of N applications have been proposed, this exposes plants to nutritional imbalances caused by unclear models of plant-pathogen interactions, which compromise available forms of N for the host or microorganism (Huber & Watson, 1974). The characteristics of pathogens influence epidemiological processes, where obligate parasites require assimilates provided directly from living cells, whereas facultative semi-saprophytic parasites prefer senescent or toxin-releasing tissue to damage or kill host plant cells (Gupta et al., 2017).

In the case of the effect of P, positive results have been reported (Zainuri et al., 2001) for sorghum (Omondi Were & Onyango Ochuodho, 2012) and avocado (Bosse et al., 2013) and related to the reduction of diseases caused by *C. gloeosporioides*. Despite these findings, its excess increases the attack of pathogens such as *Fusarium oxysporum* f. sp. *conglutians*, *Plasmodiophora brassicae*, *Puccinia graminis*, *Thelaviopsis basicola*, *Diplodia zeae*, *Cronartium fusiforme*, *Sclerotium oryzae*, and *Septoria tritici*, among others (Prabhu et al., 2007), results that coincide with those obtained in the present investigation. The line with the above, the effect of P in the reduction of diseases is attributed to physiological processes that lead young tissues to infections due to the maturation of organs. However, the effect is not noticeable when it is observed in susceptible and/or moderately resistant cultivars (Prabhu et al., 2007), which coincides with the results obtained in the present investigation, considering the high pathogenicity of the strain and the susceptibility of the genotype used, as well as reports on the influence of this nutrient in the increasing of diseases caused by various pathogens.

Ca results showed a positive effect of high dose of this element on increase Sev. Despite the recognition of Ca as an effective element in the management of phytopathogenic microorganisms (Hawkesford et al., 2012; Madani et al., 2014; Stošić et al., 2014) and considering that *Colletotrichum* species develop their appressoria in response to physicochemical signaling of its host tissues, the results of the present research coincide with what is mentioned by Ahn et al. (2003) and Uhm et al. (2003), who demonstrated greater development of the *C. gloeosporioides* with the external application of Ca, response possibly attributed to the influence of this element on early transduction signals and the activation of genes mediated by the calcium/calmodulin (Ca/CaM) complex that induces increases in the germination and formation of conidia.

The positive response of LA and CCI could be translated into maximum biomass production and physiological indices with the maximum dose of N and the application of the lowest dose of Ca. These results agree with reported by Cardona et al. (2018) and Qiang et al. (2019), the former authors evaluated the effect of the concentration of N on LA in Andean blackberry and maize (*Zea mays* L.). They observed a significant increase of LA when the concentration of N was increased and didn't report significant interaction of N and Ca in LA. The largest leaf dry matter in Andean blackberry plants with thorns was reached with a maximum dose of N and CaO lowest doses per plant.

Tissues that have chlorophyll as protein complexes, and when N is deficient, the loss of the chloroplast structure is commonly observed (Barker & Bryson, 2006) and, consequently, causing the decrease in crop growth and yield under various stress conditions (Song et al., 2019; Wamalwa et al., 2019). Nutritional imbalances can be caused by high concentrations of Ca in the root system, producing less potential for root regeneration and a low index between the aerial and total dry weight. This response can be attributed to the numerous functions of Ca, among those that stand out the interaction with phytohormones, enzymatic activities, and effect on the structure of cell walls and membranes are mentioned (Pilbeam & Morley, 2007). According to its functionality, when this element is found excessively in the rhizosphere solutions, tissue toxicity can occur, as well as low to no germination of seeds and decreased growth rates in plants (Chondraki et al., 2012; Zhang et al., 2019).

In the case of ICC and P, it was observed a negative effect on this variable whit the maximun dose of P, which differs with that reported by Meng et al. (2021) and Thuynsma et al. (2016), who mention that the deprivation of this element causes a marked decrease in photosynthetic relationships, in addition to respiration and a decrease in plant biomass.

In the present investigation, PLH and StD reached the highest values with the highest doses of N. These results differ from those reported by some authors in terms of the increase in PLH when N was applied together with P and K (Cardona et al., 2018; Close et al., 2005; Xiang et al., 2012), since the PHL increase occurred only by the application of N and not jointly with other nutrients. The plants treated with high doses of N achieved higher heights than those registered with the other evaluated doses which agree with the results of Cardona et al. (2018). Lack of N prevents cell elongation and division (Luo et al., 2017), and N is part of several crucial compounds for metabolism, and is an essential macronutrient associated with transcriptional regulation of the plants (Gaudinier et al., 2018).

The decrease in StD could also be influenced by a nutrient imbalance in the applied solutions (salinity due to excess of accompanying anions) that contemplated a high concentration of cations. Therefore, the water content in the stems could be affected, and the decrease dry matter accumulation and tissue turgor could be generated, as mentioned by Cardona et al. (2017). Plants subjected to saline stress showed significant decreases in their growth rates and water absorption (Munns, 2002), and *R. glaucus* species don't tolerate high sodium concentrations or salinity conditions (Cardona et al., 2017; Casierra-Posada & Hernández (2006).

According to the results of plant growth variables (PLH, CCI, LA, and DW) in PC1, the amount of N absorbed reflects the essentiality of this nutrient for the synthesis of many cell components. Meanwhile, adequate concentrations of Mn contribute to the production of dry matter, an increase in photosynthesis, and the chlorophyll content, as well tolerance of freezing temperatures and a decrease of the development rate of fungal diseases (Broadley et al., 2012).

The negative effect of Ca, Mg, Na, and Cu on the growth variables can be explained by the antagonism and/or reaction between the cations of the nutritional solutions used in the treatments that contemplate major cationic imbalances and in consequence, cause salinity. Andean blackberry plants exposed to saline stress reduced water consumption and photoassimilates production, essential processes in biomass production (Cardona et al., 2017).

Conversely, the highest concentrations of S, Fe, Zn, and P observed in PC2, have effect at the physiological level (Broadley et al., 2012; Hawkesford et al., 2012) and represented a positive effect at the phytosanitary level when they are in balanced concentrations. S has been recognized throughout history in disease control for its fungitoxic effect that inhibits spore germination and hyphal growth (Williams et al., 2002).

Fe can increase local oxidative stress as a defense response against pathogens. While Zn contributes to the integrity of biomembranes and reduces the leakage in the membrane of low molecular weight compounds that become food substrates for pathogens (Gupta et al., 2017). P has a role in disease resistance which is inconsistent and apparently unpredictable, depending on the type of pathosystem.

The higher severity of the disease (Sev) and its negative correlation with the stem diameter (StD) observed in PC3, understanding as the reduction of the diameter when the severity of the disease increases, is supported by the correlation analysis of this epidemiological variable with the allometric variables (Table 3), as well as the possible salt stress due to high concentrations of this cation in some treatments that can cause reductions in growth rate and water absorption.

Conclusion

Combined dose of 55 g of N, 3 g of P_2O_5, 14 g of K_2O, and 9 g of CaO caused lower severity percentage and disease rate development, as well as the highest records in the growth variables.

The suggested fertilization and applied for 90 days in the nursery phase, allows plants to tolerance to anthracnose and contributes to the establishment of plants sanitary in the field.

It is suggested to pay attention to the importance of managing the dose of Ca because under the conditions of this investigation a maximum level of response was obtained with the application of 9 g plant of CaO.

The fertilization recommendation could be extrapolated and validated in field studies where the pathogen pressure and atmospheric humidity conditions are high, to corrobotate this efficiency in anthracnose severity.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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